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ANAEROBIC CULTURES IN SCARLET FEVER *

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Plate I

The association of streptococci with scarlet fever has been so constant a finding as to be suggestive of an etiological relationship. It seemed, however, desirable to make a search for organisms by newer cultural methods, those which have usually been used being poorly adapted to the culture of many bacteria. Hence blood-cultures were made by different anaerobic methods in a series of 24 cases. Only 4 of these cultures were negative. As soon as it was found that organisms were obtainable in this way, cultures of the throat and urine were made by similar methods. In addition to these cultures opportunity was afforded by two autopsies to obtain growths from the tissues of the lymph glands, spleen and kidney. The methods of culture used were as follows:

Blood Cultures.—From 10 to 15 c.c. of blood were taken and cultures were made in different ways. The blood was usually divided into two parts. To one part were added 1-2 volumes of 1 per cent sodium citrate in normal salt solution. The other part was made into anaerobic plates according to the method described by Krumwiede.[1]

According to this method the fresh blood was added to tubes of melted agar at 39-40 C. in the proportion of about 1 c.c. to about 9 c.c. of agar. After the blood and agar were thoroughly mixed, the mixture was poured into an inverted top of a Petri plate, and the bottom, also inverted, laid directly on the melted agar. As soon as the agar solidified, the space between the rims of the two parts of the plate and over the exposed agar was sealed with boiling paraffin. From 6 to 12 of these plates were made and incubated at 35-37 C. Plates were allowed to incubate for a month or more before they were considered sterile, but for the most part growths did not appear after two weeks. The technic of making the anaerobic plates was varied in three ways. In most cases ascites fluid was added to the agar and also bits of sterile rabbit kidney or spleen. In some cases the entire quantity of blood taken from the patient was citrated and the plates made from the citrated blood.

With citrated blood, cultures were usually made in one of two ways. The corpuscles were allowed to settle by simple gravitation at room temperature. At the end of an hour the clear citrate plasma was pipetted off and cultures made by adding the fluid to melted ascites agar in tubes with a bit of sterile rabbit kidney at the bottom. After the agar solidified, the tube was made anaerobic by adding sodium hydrate and pyrogallic acid to the cotton plug and sealing the tube

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[1] *Jour. Infect. Dis.*, 1913, 12, p. 199.

with a paraffined cork. In other cases the citrated blood was centrifugalized at about 3,000 revolutions per minute for one-half hour, and then, after being decanted and having sterile distilled water added to it, was centrifuged in the same way again. By repeating this procedure the hemoglobin was eliminated and cultures made in the same way from the sediment as from the citrated plasma.

Cultures from the organs at autopsy were made by cutting out blocks of tissue with sterile instruments, searing the surface by immersion in boiling water, and macerating in a mortar. The liquid thus obtained was spread over the surface of ascites blood agar slants and added to tubes of ascites agar with rabbit kidney and made anaerobic. Cultures from catheterized urine were made by centrifugating 25-50 c.c. of urine for one-half hour at about 3,000 revolutions per minute and making cultures from the sediment in the same way as from the blood sediment.

Subcultures were made on anaerobic ascites goat blood agar slants from everything in the plates and tubes resembling colonies. Direct smears were also made at times but it was difficult in many cases to decide from direct smear whether or not organisms were present in what appeared to be a colony, inasmuch as some organisms were small, pleomorphic, few in number, and hard to distinguish from artefacts. In the plates, growths extending in from the edges were regarded as contaminations and were not examined. As a rule there developed from the 10-15 c.c. of blood used only a few colonies.

Cultures from the throat were made by shaking off the particles from swabs of the tonsils into plain broth and making cultures from this in serial dilutions as from the fluid obtained from organs. The throat cultures were made after a number of cultures from other sources were grown, with the idea of obtaining evidence as to the source of the organisms found in other parts of the body.

A brief description of the organisms found is given in the tables. A few of the throat cultures are tabulated in order that the organisms found may be compared with those in the other tables.

The results of the examination of the spleen, lymph glands and pericardial fluid of Case 1 follow. The pericardial fluid gave in the Noguchi cultures a peculiarly shaped organism (see Fig. 1, Plate 1), which developed in five days, and in two weeks a white snowball-like colony formed. The organism was strictly anaerobic and grew only on blood or ascites media. The spleen gave (*a*) a hemolytic streptococcus, (*b*) an anaerobic pigment-forming bacillus growing in jet-black colonies, (*c*) long threadlike organisms growing only anaerobically and (*d*) diphtheroid bacilli. The cervical lymph glands gave (*a*) a hemolytic streptococcus, (*b*) a non-hemolytic streptococcus growing best anaerobically, (*c*) a fine pleomorphic Gram-negative bacillus, and (*d*) long threadlike anaerobic Gram-negative organisms. The axillary and inguinal lymph glands yielded no growths.

In Case 2 the spleen was not examined. The axillary and inguinal lymph glands were sterile; aerobic cultures of the cervical lymph glands yielded a hemolytic streptococcus.

A comparison of the organisms obtained from the blood, throat, urine and of the organs at autopsy shows that, to a great extent, the same organisms are found in all of them.

A discussion of the organisms which, on account of the frequency of their occurrence in this series are of particular interest, follows. Their pathogenic powers were tried usually on guinea-pigs, but also on dogs and rabbits from which the hair was removed with potassium sulphid in order that rashes might be observed.

1. *Small Pleomorphic Gram-Negative Organisms.* — Occasionally Gram-positive or doubtful, these organisms resemble cocci so closely at times that they are hard to distinguish from cocci without some study; they vary somewhat in size, but as a rule are about the length of an influenza bacillus though more slender; they are motile to a varying degree. In the anaerobic cultures the growth usually appears in from one to two weeks as a fine pin-point-like colony which reaches the size of a millimeter or more in diameter. On the surface of blood agar slants these organisms form delicate, transparent, pin-point colonies. After subcultures have been made for a time, the organisms grow to a varying extent aerobically and after a number of months a comparatively profuse growth may be obtained in from twenty-four to forty-eight hours, the organisms growing to some extent on plain media also. In 7 of the 20 positive cultures these organisms were found in the blood. They were isolated from the throat in pure culture in 3 cases, from the kidney in both cases examined; from the urine in 3 of 6 cases examined, and from the lymph glands and spleen at autopsy. These organisms vary in pathogenic effect, but as a rule are fatal for guinea-pigs in the small doses used. They cause death after subcutaneous injection in from twelve to twenty-four hours. In one case a localized erythema appeared a few minutes after the injection. Agglutination tests were made with all the organisms of this type which were isolated, convalescent scarlet fever serum being used, and as a control, serum from convalescent diphtheria patients. The results follow:

1–3.	No agglutination.	
E–3.	Scarlet serum 1–40	Control 1–80
O–2.	Scarlet serum 1–160	Control 0
V–1.	Scarlet serum 0	Control 0
H–2.	Scarlet serum 0	Control 0
B–1.	Scarlet serum 0	Control 0
II–2.	Scarlet serum 1–40	Control 0

TABLE 1

ORGANISMS FOUND IN BLOOD

Case	Morphology	Gram	Aerobic or Anaerobic	Blood	Litmus Milk	Litmus Dextrose	Litmus Mannite	Litmus Inulin	Litmus Lactose	Plain Agar	Character of Growth
A	1. Small pleomorphic bacillus, motile	±	Anaerobic	No effect	No growth						Very small, transparent colonies
B	1. Small pleomorphic bacillus, tumbling motility	+	"	" "	No growth						Small, transparent, round colonies
C	1. Small streptococcus in short chains	+	Aerobic	" "	Slightly Acid	Acid	Acid	—	Acid	Fair growth	Small, round, white, opaque colonies
	2. Spore-forming bacillus	+	"	" "	....	....	....	....	....	Good "	....
	3. Small pleomorphic bacillus	—	Anaerobic	" "	—	—	—	—	—	Fair "	Small, transparent colonies
D	1. Diphtheroid bacillus	+	Aerobic	" "	—	Acid	—	—	—	Good "	Grayish, translucent film
E	1. Streptococcus in fairly long chains	+	......	Hemolysis	....	....	....	....	....	....	....
	2. Pigmented pleomorphic organism	—	Anaerobic	"	No growth						Black, convex, fairly large colonies
	3. Small pleomorphic bacillus, motile	—	"	....	....	....	....	....	....	No growth	....
	4. Small staphylococcus	±	....	....	....	....	....	....	....	....	No growth
F	1. Spirillum	—	Anaerobic	—	No growth						Pin-point, transparent colonies
G	1. Diplococcus	+	Aerobic	....	Acid	—	—	—	—	Good growth	Moist, grayish, semi-transparent film
	2. Diplostreptococcus of varying size	+	"	Brown hemolysis	Acid	Acid	Acid	Acid	Acid	" "	White, moist, translucent film
	3. Small streptococcus	+	"	—	Acid	—	—	—	....	" "	Moist, yellowish film
	4. Short, plump bacillus with rounded ends	+	"	Green hemolysis	—	Acid	....	....	....	" "	Small, transparent colonies
	5. Spore-forming bacillus with square ends, motile	+	"	"	Coag., bleached	"	Acid	Acid	—	" "	Dry, heavy, white, adherent growth
	6. Biscuit-shaped diplococcus	—	"	Slight hemolysis	—	—	—	—	—	" "	Lemon-yellow, moist, opaque
H	1. Small diplostreptococcus	+	"	Green brown	Acid	Acid	—	—	....	" "	White, translucent film
	2. Spore-forming bacillus with square ends	—	"	Hemolysis	Pept. bleached	Bleached	Bleached	Acid	....	" "	Heavy, white, dry, adherent film
	3. Staphylococcus	+	"	Green	Acid, coag.	Acid	—	"	Acid	Fair "	Delicate, translucent film
I	1. Spore-forming bacillus with rounded ends	—	"	Hemolysis	Bleached, coag.	Gas, acid	Acid	"	—	Good "	Cream-colored, opaque, moist film
	2. Oval streptococcus, rather large	+	"	"	Acid	Acid	—	—	—	Fair "	Small, round, transparent colonies
	3. Staphylococcus	+	"	"	Acid, coag.	"	Acid	—	Acid	Good "	Opaque, white smear
	4. Square end, spore-forming bacillus in chains	..	"	"	Peptonized	"	—	—	—	" "	Thick

TABLE 1.—(*Continued*)

J	1. Spore-forming bacillus	+	Aerobic	Hemolysis	Bleached, coag.	Bleached, acid	Acid	—	—	Good growth	Dry, white, opaque film, very adherent
	2. Short, plump bacilli with rounded ends; spores?	—	“	“	—	Acid	—	—	—	“ “	Thick, moist, opaque, dull-yellow film
	3. Staphylococcus	+	“	“	Acid	—	—	—	—	“ “	Opaque, white film
	4. Small bacillus, pleomorphic	—	“	“	—	—	—	—	—	Fair “	Delicate, moist, translucent film
	5. Small streptococcus	..	“	....	....	....	....	....	....	No “	Very small, transparent colonies
K	1. Small coccus	+	“	Hemolysis	Bleached, acid	Acid	—	—	....	Good “	Profuse growth of sharply outlined, yellow colonies
	2. Motile threadlike organism	—	Obl. anaerobic	—	—	—	—	—	—	Slight “	Small, transparent colonies
L	1. Short, plump bacillus	—	Aerobic	—	—	Acid	—	—	—	Good “	Moist, semitransparent film
M	1. Welch bacillus	+	Anaerobic	Hemolysis	Coag., acid	Gas, acid	—	—	Acid	Poor “	Rather large, dew-drop-like colonies
N	1. Streptococcus	+	Aerobic	—	—	Acid	—	—	—	Fair “	Transparent film
	2. Diplococcus, fairly large	+	“	—	—	“	Acid	—	—	Good “	Pearly white, round colonies
O	1. Large diplococcus	+	“	....	Slightly acid	“	—	—	—	“ “	Orange-colored
P	1. Spore-forming bacillus	+	“	Hemolysis	Pept., bleached	Bleached	Bleached	—	....	“ “	Dull-white, moist film, not adherent
	2. Spore-forming bacillus	+	“	“	—	Acid	—	—	—	“ “	Translucent film
	3. Small streptococcus, short chains	+	“	“	Coag., acid	“	Acid	—	Acid	“ “	Moist, white, translucent film
	4. Slender bacillus	—	....	....	....	....	....	....	....	....	....
	5. Spore-forming bacillus in filaments	—	Aerobic	Hemolysis	Coag., bleached	Bleached	—	—	—	Good growth	....
Q	1. Large diplostreptococcus	+	“	....	....	....	—	—	—	“ “	Wrinkled, moist film
	2. Large spore-forming bacillus	+	“	....	Acid	Acid	Acid	Acid	—	“ “	....
R	1. Threadlike organism	—	....	....	....	....	....	....	....	....	Not grown in pure culture
S	1. Small pleomorphic bacillus	—	Anaerobic	—	....	....	....	....	....	....	No growth
T	1. Streptococcus	+	Aerobic	Hemolysis	Coag., acid	Acid	....	—	Acid	Good growth	White, gradually becoming orange
	2. Small pleomorphic bacillus	±	Aerobic and anaerobic	Slight hemolysis	—	—	—	—	—	“ “	Cream-colored translucent film

TABLE 2

ORGANISMS FOUND IN THROAT

Case	Morphology	Gram	Aerobic or Anaerobic	Blood	Litmus Milk	Litmus Dextrose	Litmus Mannite	Litmus Inulin	Litmus Lactose	Plain Agar	Character of Growth
O	1. Streptococcus, irregular in size, short chains	+	Aerobic and anaerobic	Hemolysis	Acid	Acid	No effect	No effect	Acid	Fair growth	Delicate, transparent
	2. Small pleomorphic bacillus, motile	—	"	No effect	No growth					Slight "	Small, transparent colonies
	3. Small diplostreptococcus	—	Anaerobic	" "	No effect	Acid	No effect	No effect	No effect	Fair "	Delicate, transparent
	4. Wavy, threadlike organism	—	....	....	....	....	....	....	....	....	No growth. Not obtained in pure culture
	5. Fusiform bacillus	—	Anaerobic	....	....	....	....	....	....	....	No growth. Not obtained in pure culture
G	1. Diplococcus	—	Aerobic and anaerobic	No effect	Acid	Acid	No effect	No effect	No effect	Fair growth	Delicate, transparent, round colonies
	2. Streptococcus in fairly long chains	+	Aerobic	Hemolysis	"	"	" "	" "	Acid	" "	Delicate, transparent, round colonies
	3. Coccus occurring singly, in pairs, chains, and clusters	±	Aerobic and anaerobic	"	"	"	" "	Acid	No effect	Good "	Small, hemispherical sharply outlined lemon-yellow colonies
	4. Rather small streptococcus	..	Aerobic	"	No effect	"	Acid	No effect	" "	" "	Moderately thick, whitish film
	5. Curved threads of varying length with bacillary forms	—	Anaerobic	No effect	No growth					Slight "	Small, transparent colonies
	6. Pigmented pleomorphic organism	—	"	Hemolysis	No growth						Large, convex, black colonies hard to grow
	7. Slender bacillus with terminal spores	—	"	....	Faintly acid	Acid	Acid	....	Faintly acid	Slight growth	Slight growth except on dextrose agar
	8. Slender bacillus	—	Aerobic	No effect	No effect	No effect	No effect	No effect	No effect	Good "	Fairly heavy, semi-transparent, moist film

TABLE 2 —*Continued*

H	1. Very small streptococcus with many gram-individuals	+	Aerobic and anaerobic	Hemolysis	Coag., acid	Acid	—	Acid	Acid	Fair growth	Delicate, transparent
	2. Small pleomorphic bacillus, motile	+	Anaerobic	No effect	No effect	No effect	No effect	No effect	No effect	“ “	Very thin, transparent
	3. Fusiform bacillus	—	“	“ “	“ “	“ “	“ “	“ “	“ “	Slight “	Large, round, semi-transparent, coherent colonies
	4. Diphtheroid bacillus	+	Aerobic	“ “	“ “	“ “	“ “	“ “	“ “	Fair “	Very delicate, transparent
P	1. Small pleomorphic bacillus	+	Anaerobic	“ “	Acid	“ “	“ “	“ “	“ “	Slight “	Very small, transparent colonies
	2. Very small pleomorphic bacillus	—	Aerobic and anaerobic	Hemolysis	“	Acid	“ “	“ “	“ “	Fair “	Very small, transparent colonies
	3. Moderately large diplococcus	+	Aerobic and anaerobic	Green hemolysis	“	“	“ “	“ “	“ “	“ “	Thin, transparent film best on dextrose
	4. Pigmented pleomorphic organism	..	....	Hemolysis	No growth						Black, raised, round colonies
F	1. Cladothrix	—	Aerobic	“	No effect	No effect	No effect	No effect	No effect	Good growth	Large, round, convex, adherent, white colonies
	2. Very small streptococcus	+	“	Slight green	Coag., acid	Acid	“ “	“ “	“ “	Slight “	Delicate, transparent, small, round colonies
	3. Fairly large streptococcus	+	“	No effect	No effect	No effect	“ “	“ “	“ “	Good “	Reddish-orange colored film
	4. Diplostreptococcus, small	+	“	Hemolysis	“ “	Slightly acid	“ “	“ “	Slightly acid	“ “	Heavy, orange-yellow film
	5. Staphylococcus	+	“	“	“ “	No effect	“ “	“ “	No effect	“ “	Thick, moist, lemon-yellow film
	6. Diphtheroid bacilli	+	“	No effect	“ “	“ “	“ “	“ “	“ “	Fair “	Delicate, round, transparent colonies
	7. Spore-forming bacillus	+	“	Hemolysis	Coag., bleached	Acid	“ “	Acid	“ “	Good “	Heavy, white, dry, very adherent film
	8. Pigmented pleomorphic organism	—	Anaerobic	“	No growth						Black, round colonies

TABLE 3

ORGANISMS FOUND IN URINE

Case	Morphology	Gram	Aerobic or Anaerobic	Blood	Litmus Milk	Litmus Dextrose	Litmus Mannite	Litmus Inulin	Litmus Lactose	Plain Agar	Character of Growth
D	1. Somewhat long, slender bacillus resembling B. tuberculosis	—	Aerobic	Green	—	Bleached	—	—	—	+	Moist, somewhat wrinkled film, whitish, turning yellow later
	2. Small pleomorphic bacillus	—	Aerobic and anaerobic	"	Slight bleaching	Acid	—	—	—	—	Moist, gray, translucent film; old colonies yellow
	3. Small pleomorphic bacillus. Same as D2?	—	"	—	Slightly acid	—	—	—	Slightly acid	+	Delicate transparent growth
	4. Spore-forming bacilli with many bizarre forms	+	Aerobic	Green hemolysis	—	Bleached	—	Acid	—	+	Rather thick, almost transparent film
	5. Pleomorphic pigmented organism	—	....	....	....	....	....	....	....	....	Not obtained in pure culture
	6. Wavy, threadlike organisms	—	....	....	....	....	....	....	....	....	Not obtained in pure culture
G	1. Ordinary staphylococcus	+	Aerobic	....	Acid, not coag.	Acid	Acid	—	Acid	+	Moist, semitranslucent, grayish film
	2. Spore-forming bacillus slightly larger than B. coli	—	"	Brown hemolysis	Coag., bleached	"	"	Acid	—	+	Heavy, opaque wrinkled, brown film
	3. Streptococcus. Medium long chains	+	"	Hemolysis	—	"	—	—	—	+	Delicate, transparent growth
	4. Vibrio—larger and more slender than comma bacillus	—	"	"	Acid	"	—	—	—	+	Yellowish, transparent film
	5. Pleomorphic pigmented organisms	—	....	....	....	....	....	....	....	....	Not grown in pure culture

TABLE 3 —*Continued*

N	1. Diplococcus resembling Dip. catarrhalis	—	Anaerobic	—	—	....	Bleached	—	+	—	Heavy white, opaque growth
	2. Streptococcus in short chains	+	Aerobic	—	—	Acid	Acid	—	Acid	+	Heavy brownish growth
	3. Diphtheroid organism	+	....	....	....	....	....	....	....	....	Not grown in pure culture
V	1. Very small pleomorphic bacillus, motile	+	naerobic	—	No growth		....	....	....	Slight	Delicate, transparent growth
	2. Round diplococcus about size and shape of ordinary staphylococcus	+	"	—	Acid, coag., bleached	Acid, no gas	—	—	Acid	Good growth	Transparent film
	3. Long, wavy, threadlike organisms	—	....	....	....	....	....	....	....	....	Not grown in pure culture
	4. Pigmented bacilli, pleomorphic	—	....	....	....	....	....	....	....	....	Not grown in pure culture. Round, convex, black colonies
X	1. Very small pleomorphic bacilli	—	....	....	....	....	....	....	....	....	Not grown in pure culture
Y	1. B. coli morphology	+	Aerobic	....	....	....	....	....	....	....	No growth
	2. Diplococcus-gonococcus morphology	—	"	....	....	....	....	....	....	....	No growth
	3. Influenza-like bacilli	—	Anaerobic	....	....	....	....	....	....	....	....
Z	1. Spore-forming bacillus slightly larger than coli	—	Aerobic	Hemolysis	—	Acid	Acid	Acid	Acid	+	Moist, luxuriant growth
	2. B. coli	—	"	—	Acid cong.	"	"	—	"	+	....
	3. Pigmented pleomorphic organisms	—	Anaerobic	Hemolysis	....	....	....	....	....	No growth	Round, convex, black colonies

TABLE 4

ORGANISMS FOUND IN KIDNEY

Case	Morphology	Gram	Aerobic or Anaerobic	Blood	Litmus Milk	Litmus Dextrose	Litmus Mannite	Litmus Inulin	Litmus Lactose	Plain Agar	Character of Growth
I	1. B. coli	—	Aerobic	—	Coag., acid	Acid, gas	+	—	+	+	....
	2. Staphylococcus	+	"	—	....	....	....	....	....	....	....
	3. Small pleomorphic bacillus, motile	—	Anaerobic	....	....	....	....	....	....	....	No growth
	4. Long, wavy, threadlike organisms	—	"	....	....	....	....	....	....	....	No growth
II	1. Bacillus similar to B. coli	+	"	....	....	....	....	....	....	....	No growth
	2. Small pleomorphic bacillus, motile	—	"	....	....	....	....	....	....	....	No growth
	3. Long, wavy, threadlike organisms	—	"	....	....	....	....	....	....	....	No growth
	4. Very fine coccus	±	"	—	Coag., alk.	Alk.	—	—	Alk.	Slight	Very fine dustlike colonies
	5. Pigmented pleomorphic organism	—	"	Hemolysis	....	....	... .	....	....	....	....

It will be seen that the results of the agglutination tests are irregular and somewhat contradictory. In two cases, however, there was more agglutination with scarlet serum than with normal serum. The test is of little significance, as the presence of the organism even as a secondary infection might result in agglutinin formation.

2. *Pigment-Forming Organisms.*—Such organisms are not infrequently described in connection with various pathologic processes, including appendicitis, lung abscess, renal infections, etc. They are also of protean morphology, for the most part, however, small bacillary forms shorter than colon bacilli. They appear in the cultures after a few days as pin-point, semi-transparent colonies which gradually absorb coloring material from blood or tissues and become a tarlike mass of jet-black pigment. They were found in the blood, the spleen and the kidney in the two autopsies; in the urine of 4 cases of 7 examined. They were also found in the throat in many more cases than are represented in the table. In the throat cultures of chronic tonsillitis, however, they were quite as common. They are toxic for guinea-pigs and cause death in from twelve to twenty-four hours, according to the dose used, but produce no lesions suggestive of scarlet fever.

3. *Long Threadlike Organisms.*—These organisms were exceedingly difficult to isolate in pure culture and the only one which was obtained in any quantity for experimental work was from the blood. It grew slowly, taking a number of days to develop; after a considerable time it grew also on Löffler's blood-serum and ordinary media. It was at first anaerobic, but later grow aerobically. After a long period of growth, however, the organism took on a morphology more like that of the diphtheria bacillus than its original threadlike form. It was very toxic for guinea-pigs and on intravenous injection caused almost immediately subcutaneous petechial hemorrhages and in a few minutes death with convulsions. The subcutaneous injection resulted in several animals in petechial rashes and death in from twelve to twenty-four hours. The organism was agglutinated by scarlet serum in a dilution of from 1 to 40 and by diphtheria serum in a dilution of from 1 to 80.

4. *Spore-Forming Bacilli Somewhat Like Bacillus Subtilis.*—This grew best aerobically, the growth being very adherent to the media. The organisms were regarded at first as contaminations, but a record was kept of their occurrence. Later, however, they were isolated from

the throat, and in the examination of direct smears from the throat organisms of this shape occur in a high percentage of cases. The injection subcutaneously of a considerable quantity of these organisms into a guinea-pig resulted in a diffuse generalized erythema in about twenty-four hours, followed a few days later by a desquamation resembling that of scarlet fever. It was accidentally found during an attempt to obtain growths from throat mucus passed through Berkefeld filters that these organisms pass through the filter much more readily than do smaller organisms. This may be due to the minute spores which were later demonstrated with ordinary stains for spores. It is difficult to interpret the animal experiments. They were repeated a number of times and rashes obtained in some cases and not in others.

5. *Hemolytic Streptococci.*—These organisms were found in the blood in 7 of the 20 positive cases, in the lymph glands in 2 cases, in the spleen in 2 cases and in the urine in 3 cases. These results are simply confirmatory of those of a large number of other observers.

6. *Very Small Streptococci.*—In 5 cases such organisms were isolated from the blood. They were much smaller than the hemolytic streptococci, did not as a rule affect blood and occurred very often in clusters as well as chains.

7. *Gram-Negative Diplococci* — These organisms were recovered from the blood in 1 case, from the urine in 2 cases, and from the throat in 1 case. Injection of the organism from Case G-6 resulted in a rash closely resembling that of measles. At autopsy the kidneys showed a marked cloudy swelling.

8. *Large Gram-Positive Diplococci.*—These were found in the blood in 3 cases, in the urine in 2 cases and in the throat in 1 case. The pathogenic effect on animals was not tested.

9. *Diphtheroid Bacilli.*—These organisms were recovered from the blood in 1 case, from the urine in 1 case and from the throat in 2 cases. No tests were made on animals to determine whether they were true diphtheria bacilli or not.

It will be seen from the tables that a number of other organisms, including *B. welchii,* were recovered from the blood. One of the most interesting of the organisms found was the spirillum in Case F. The organism was found only in one blood-culture, but it grew in very minute colonies which were hard to find, and even had it been present in the blood in a high percentage of cases it might readily have been overlooked. In a series of mixed cultures made by simply inoculating ascites broth from the mucus from scarlet angina, spirilla closely resembling the one recovered from the blood were found in nearly all of them. They were also found, however, in one of four controls from the throats of diphtheria cases. Cutaneous tests with killed cultures of this organism in scarlet convalescents gave no decisive results.

SUMMARY AND CONCLUSIONS

In blood-cultures from 24 cases made anaerobically, positive results were obtained in 20 cases.

A comparison of the kind of organisms found in these blood-cultures with the organisms present in the throat and urine would indicate that during the acute stage of scarlatinal angina, organisms enter the blood-stream in considerable number and are excreted in part at least in a viable condition through the kidneys.

The excretion of living bacteria through the kidney may have some connection with the development of scarlatinal nephritis.

A number of organisms are described which are commonly associated with scarlet fever, some of which produce rashes in guinea-pigs, but it is not possible to say that any of these animals really had scarlet fever.

EXPLANATION OF PLATE 1

FIG. 1.—Spirillum from pericardial fluid (Case 1). X 1200.

FIG. 2.—Spirillum from blood (F, Table 1). X 1200.

FIG. 3.—Pleomorphic bacillus from the urine (D2, Table 3). X 1200.

FIG. 4.—Pigment-forming bacillus from the throat (H2, Table 2). X 1200.

FIG. 5.—Bacillus from the blood (C3, Table 1). X 1200.

PLATE 1

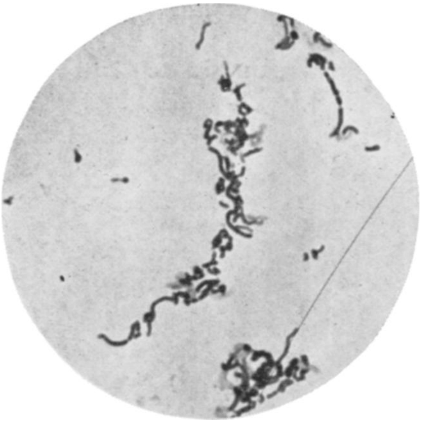

Fig. 1

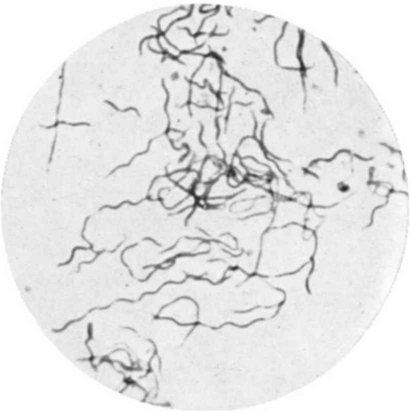

Fig. 2

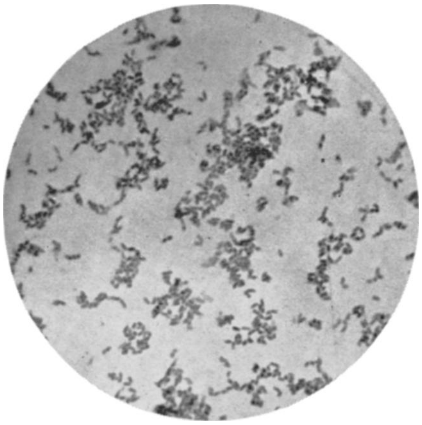

Fig. 3

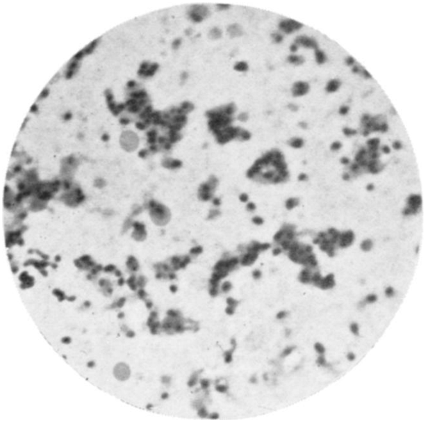

Fig. 4

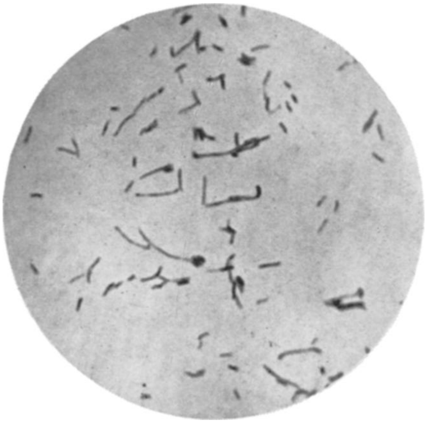

Fig. 5